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SPECTRAL SENSITIVITY OF THE COMPOUND EYES IN THE PURPLE LAND CRAB *GECARCINUS LATERALIS* (FREMINVILLE)

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Abstract

The spectral sensitivities (S(λ)) of dark-adapted compound eyes of the purple land crab *Gecarcinus lateralis* possess a broad maximum in the blue-green, 420–530 nm, when measured by electroretinographic (ERG) techniques. Selective adaptation experiments showed large changes in sensitivity but did not isolate different receptor types. A photopigment with maximal absorption at 487 nm was identified in the rhabdoms by microspectrophotometry. Besides the presence of a dominant green receptor system, the existence of a hump in the short wavelength region in S(λ) suggests the presence of a blue-sensitive system as well. It is hypothesized that two photopigments (P487 and P440) in conjunction with screening pigment(s) mediate broad visual maximum in the blue-green in the purple land crab.

Introduction

One or two receptor systems have been described in the intertidal and subtidal crabs. Behavioral evidence for color discrimination exists for portunid crab *Carcinus* (von Buddenbrock and Friedrich, 1961) and for two *Uca* species (Hyatt, 1975). This can be accounted for by the presence of different receptor types produced either by different visual pigments (Wald, 1968) or by a single pigment in conjunction with screening pigments (Leggett, 1979). To date, microspectrophotometric (MSP) studies have revealed only a single visual pigment in crabs (*e.g.*, spider crab *Libinia emarginata:* λ max 493 nm, Hays and Goldsmith, 1969; blue crab *Callinectes spidicus:* λ max 500 nm, Bruno and Goldsmith, 1974; green crab *Carcinus maenas:* λ max 505 nm, Bruno *et al.*, 1973, crab species, Cronin and Forward, 1987).

Electrophysiological studies have produced varying results regarding the types and numbers of photoreceptors in eyes of intertidal and subtidal crabs, even in the same species. Some workers using electroretinographic (ERG) or single unit recordings have located only a single receptor system. For example, Scott and Mote (1974) observed a single maximum at 510 nm in crabs inhabiting diverse habitats (*Callinectes sapidus, Sesarma reticulatum, Uca pugilator,* and *U. pugnas*). Wald (1968) reported that the subtidal *Libinia emarginata* had a single receptor type (λ max 490 nm), and Fernandez (1973) located only one system in the deep-water species *Pleuroncodes planipes* (λ max 523 nm). Similarly, Bruno *et al.* (1973) described a single 493 nm maximum in *Carcinus maenus.* However other workers have uncovered an additional, shorter wavelength receptor system in several of these species: *C. maenas* (Wald, 1968; Martin and Mote, 1982), *C. sapidus* (Martin and Mote, 1982), and *U. pugilator* (Hyatt, 1975). Thus it seems likely that crabs in general are dichromats.

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The land crabs are decapod Crustracea which have made the transition from water to land. During their evolutionary adaptation to a new niche, a selection of a new set of physiological and behavioral mechanisms differing from their ancestors would be expected which could produce alterations in their visual systems. The visual pigments of several intertidal crab species have been described (Cronin and Forward, 1987), and some semiterrestrial crab species have been investigated electrophysiologically as described in the previous paragraph. Nevertheless, the spectral characteristics of vision are not known for the purple land crab. The objective here is to fill this gap in our present knowledge. The land crabs live in burrows in the sand or in the mangroves. Most of them forage at twilight or at night in a light limiting condition.

Materials and Methods

Animals

The purple land crabs (4–6 cm carapace width) used in these experiments came from the West Coast of Florida and were kept in containers in the laboratory at room temperature (21–23°C) with a daily light cycle of 12 h light and 12 h dark.

Experimental preparation

Electrical recordings were taken from the corneal surface in live, intact preparations. The crabs were immobilized by bandaging heavily with gauze and then further secured with adhesive tape. The eye of the crab is retractable, so it was immobilized with dental cement to ensure that it remained easily accessible for experimentation. The animals were allowed to dark-adapt for one hour. All the experiments were conducted during the photophase at room temperature (21–23°C).

Electrical recordings

ERGs elicited by illumination of the eye were recorded by a glass pipette (tip diameter 5–10 μm) filled with physiological saline and inserted underneath the cornea. The reference electrode, also filled with saline, was inserted into a small hole bored in the shell of the animal. An Ag-AgCl wire made the connection between the electrodes and the grid of a high impedance preamplifier. The output of the preamplifier was fed to a DC coupled cathode ray oscilloscope (CRO) and a chart recorder.

Optical system

A two-channel optical system was utilized (details given in Lall *et al.*, 1982). One beam was for testing and other for chromatic adaptation. The test beam was obtained from a 150 watt xenon arc operated at 7.5 amps. with a regulated power supply. The light beam passed a high intensity grating monochromator. Quartz lenses collimated the test beam and focussed it on the entrance of a quartz light pipe (3×360 mm). The adapting beam was obtained from a 500 watt tungsten quartz iodide lamp, and was collimated with a quartz lens and superimposed on the test beam using a beam splitter. The quantum flux in both light beams was controlled by calibrated neutral density filters. Both beams were interrupted by Uniblitz photographic shutters which controlled the duration of the test flash and adaptation times.

Intensity calibration

The quantum flux of the test and the adaptation beam were determined by using a calibrated PIN-10UV Schottky barrier photodiode with a Model 210A amplifier

(United Detector Technology, Inc.). The photodiode was positioned directly at the end of the quartz light pipe in the same way the eye was positioned during experimentation and its output read on the CRO.

Experimental procedures

Light flashes of varying duration (0.1–0.4 s) at 25 nm or 20 nm steps from 340 to 680 nm were administered over 5 log units of intensity change. The ERGs elicited by these flashes were recorded. To maintain a constant steady dark-adapted state, the test flashes were delivered at least 30 s to 90 s apart depending upon the intensity of the test flash.

Spectral sensitivity curves

The amplitude of the ERG was used as an index of the sensitivity of the eye to the quantum flux and the wavelength composition of the photic stimulus. The spectral sensitivity functions were obtained by first determining the number of photons needed to elicit a criterion amplitude of the ERGs for different stimulus wavelength. A plot of 1/Q as a function of wavelength gave the spectral sensitivity function. Two variations of the criterion method were used for determining the spectral sensitivity curves. (a) ERGs were recorded at different levels of intensity for a stimulus wavelength and V/logI function was obtained. Similar functions were obtained for all the selected stimulus wavelengths across the spectrum. The reciprocal of the quanta needed to elicit a chosen criterion amplitude response across the stimulus wavelengths, as a function of wavelength, gave the spectral sensitivity curve. This is a lengthy procedure, and in most cases a shorter one was adopted. (b) One person observed the CRO screen while another adjusted the intensity of the test flash with neutral density filters at each wavelength until the observer signaled that a criterion response (50 μV or 200 μV) had been met.

Several chromatic adaptation experiments were conducted. Either Corning glass filters: blue (Cs5-60) and orange (Cs2-73) for broad-band irradiation, or Baird Atomic Interference filters for monochromatic (440 nm and 610 nm) intense light were placed in the path of the adapting beam of the stimulator and the eye was allowed to chromatically adapt continuously during experimentation. The test flashes for determining the spectral sensitivity were superimposed on the chromatic adaptation beam. The spectral sensitivity curves under chromatic adaptation were obtained by using the second criterion method described above.

Microspectrophotometry

A single-beam instrument described in Cronin (1984) was used. Animals were dark-adapted for several days, following which eyes were removed and ground in 2.5% gluteraldehyde in pH 7.5 MBL crustacean Ringer's (Cavenaugh, 1956). After 15 minutes of fixation at 0°C, the mixture of eye debris and photoreceptors (rhabdoms) was centrifuged, resuspended in pH 7.5 Ringer's, and maintained at 0°C. Individual rhabdoms were scanned as described in Cronin (1984). The rhodopsin absorption spectrum was determined by taking the difference between the absorption of a fully dark-adapted rhabdom and the absorption of the same rhabdom after a 5 minute photobleach with bright white light. Data from 13 rhabdoms were averaged and fit with a Dartnall (1953) nomogram as described in Cronin and Forward (1987).

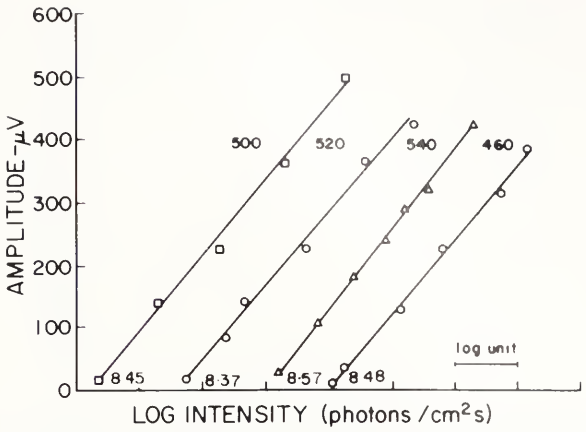

FIGURE 1. V/logI curves for a dark-adapted compound eyes in *Gecarcinus lateralis*. The number at the bottom of the curve is the log photons for the lowest response. Note that the curves for different wavelengths are similar in slope.

RESULTS

Electroretinograms (ERGs)

The ERGs were recorded from the corneal surface of the compound eyes after an initial latency period (20–60 ms) from the onset of illumination. The response was an "on" negative potential consisting of an initial phasic component followed by a maintained or plateau component which lasted for the total duration of the illumination. At low levels of illumination only the plateau component was recorded. The phasic component appeared at the intermediate levels of the illumination and increased sharply with bright illumination. The "on" negative ERGs in the land crab were similar to the ones recorded from the compound eyes of many arthropods which have scotopic eyes (*e.g.*, horseshoe crab; Chapman and Lall, 1967; Crustacea; Wald, 1968). The response waveform of the ERGs elicited by the stimuli of different wavelengths tended to be similar.

Intensity-response (V/log I) functions

Figure 1 shows the amplitude of the phasic component of the ERG plotted as a function of log intensity of the stimuli of different wavelengths and intensities. The slopes of these V/logI functions for the phasic components did not vary with stimulus wavelength. These V/logI curves were used for: (a) determining the spectral sensitivities in Figure 3 (only the DA curve) and (b) determining whether there were any wavelength-dependent changes in the slope of the V/logI functions. Systematic changes in the slopes of the response curves for different wavelengths have been taken as evidence for the presence of different receptor types as in the median ocelli of *Limulus* (Chapman and Lall, 1967) and in the compound eyes of whirligig beetle (Bennett, 1967) and wolf spider (DeVoe *et al.*, 1969). The V/logI curves extended over 4 to 5 log units of intensity change, which indicated that the photoreceptor could function over 10,000 to 100,000 fold change in stimulus intensity.

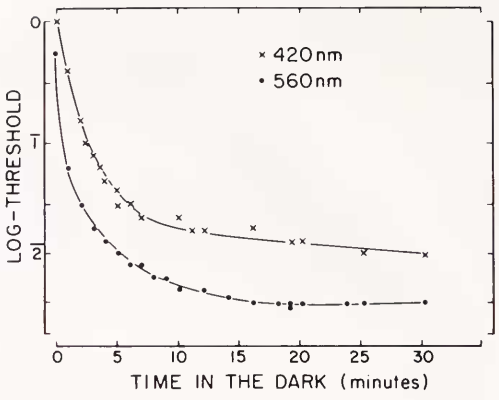

FIGURE 2. Dark-adaptation of the purple land crab compound eye measured at two wavelengths. The curves tended to be hyperbolic initially and follow a similar time course.

Dark-adaptation

Figure 2 shows the time course of dark-adaptation measured at two wavelengths (560 nm and 420 nm). The eye was light adapted for 1 minute with white light, and then the responses during dark-adaptation were tested alternately at 560 nm and 420 nm. Initially the threshold decreased hyperbolically, and then after about 20 minutes the threshold decrease was linear. These two curves are parallel, showing that the eye maintains constant relative sensitivities to the two wavelengths throughout dark adaptation.

Spectral sensitivity

Figure 3 shows the S(λ) functions under dark- and chromatic adaptation conditions in *G. lateralis*. The dark-adapted S(λ) curves showed a very broad sensitivity in the blue-green (440–520 nm) region of the spectrum. In two animals (▲, Fig. 3), the sensitivity in the blue (430–460 nm) region was pronounced. Under chromatic adaptation conditions with both Corning glass filters and narrow band interference filters, the S(λ) curves exhibited a decrease in sensitivity of about 1.5 to 2.5 log units, but a distinctive and pronounced selective effect with differential suppression of different parts of the spectrum was not observed. The chromatic adaptation curves tended to be broad and rather flat across the spectrum (compare curves a, b, and d for different chromatic adaptation conditions in Fig. 3), except for a small hump in the blue under red selective adaptation light. However, it should be noted that the ERG is a gross response from the whole eye, and chromatic adaptation experiments may poorly separate different receptor types even when the receptors are as far apart in the spectrum as near-UV and green (*e.g., Dineutes;* Bennett, 1978; *Photuris versicolor;* Lall, 1981). This difficulty in receptor isolation is further compounded when the receptors are as adjacent in the spectrum as blue *versus* green, and when the number of blue receptors is only a very small fraction of the green as in the blue crab *Callinectes* (Martin and Mote, 1982).

Microspectrophotometry (MSP)

The absorption spectrum of the visual pigment found in the rhabdoms of *Gecarcinus lateralis* is shown in Figure 4 and represents an average curve of the difference

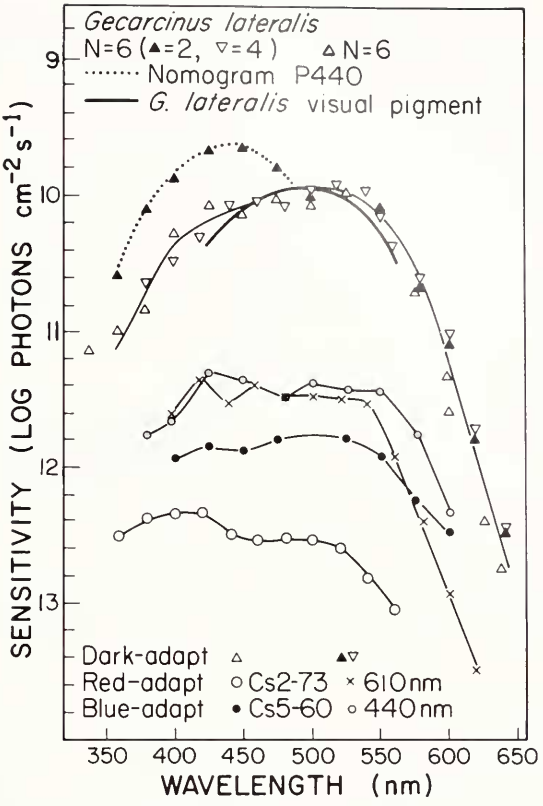

FIGURE 3. Spectral sensitivity of the purple land crab compound eyes under dark- and chromatic adaptation conditions. Nomogram (Ebrey and Honig, 1977) for P440(· · ·) and *G. lateralis* visual pigment (——) from Figure 4 are superimposed for the data.

spectra between bleaches and dark-adapted preparations of 13 individual photoreceptors. The curve possesses a peak in the blue-green. The Dartnall (1953) nomogram curve for P487 nm closely matches this difference spectrum curve (Fig. 4).

DISCUSSION

The purple land crab *Gecarcinus lateralis* possesses a primary blue-green sensitive receptor system. This is supported by the following observations: (a) similarity of the ERG waveforms as a function of wavelength, (b) univariance of V/logI slopes as a function of wavelength (Fig. 1), (c) broad dark-adaptation curves with maximum in the blue-green (Fig. 3), (d) a lack of isolation of either blue or green receptor system under conditions of chromatic adaptation (Fig. 3), and (e) the presence of a blue-green absorbing (λ max 487 nm) photopigment in the rhabdoms (Fig. 4). However this does not rule out the possibility of a second photoreceptor system, since it is possible that the contribution of a receptor type consisting of only few ommatidia can be masked by the dominant receptor system. In the retina of the swimming blue crab *Callinectes sapidus,* green-sensitive cells (λ max 508 nm) were dominant, while only a few cells restricted to the ventral border region were blue-sensitive (λ max 440

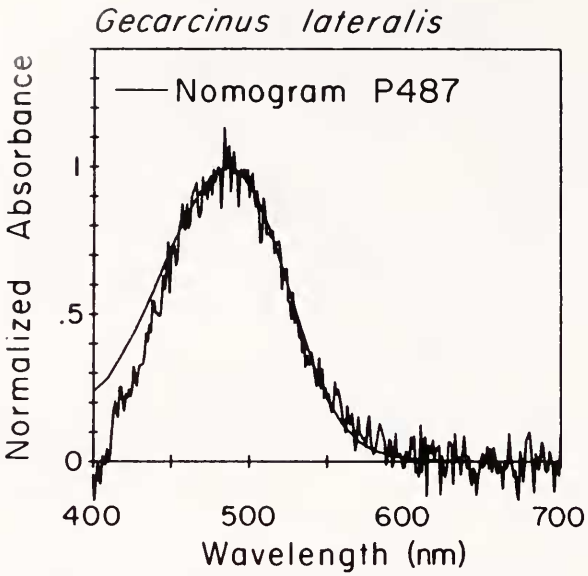

FIGURE 4. Average absorption spectrum of the rhodopsin in 13 rhabdoms determined microspectrophotometrically by taking the difference between spectra obtained from rhobdoms when dark-adapted and again when photobleached with white light. The solid line represents a Dartnall's nomogram curve for P487.

nm, Martin and Mote, 1982). Consequently, earlier studies using both single cell (Scott and Mote, 1974) and ERG (Goldsmith and Fernandez, 1968) techniques did not uncover the blue-sensitive receptor system. In our data, the presence of high sensitivity in the blue in a few recordings strongly suggests that a similar blue-sensitive receptor system (P440) exists in the purple land crab (Fig. 3).

It should be noted that the peak of the S(λ) function in *G. lateralis* is difficult to establish in our ERG data (Fig. 3). The maximal sensitivity in the blue-green is much broader than the absorption spectrum of the most prevalent visual pigment, P487 (Fig. 4), presumably responsible for the sensitivity in the green (Fig. 3). The presence of screening pigments have been implicated in modifying the visual spectral sensitivity mediated by the visual pigment(s). This modification in some Crustacea has led to a broadening of the S(λ) functions in the green (*i.e., Leptograpsus variegatus,* Stowe, 1980; *Carcinus maenas,* Wald, 1968, Bruno *et al.,* 1973). While in other species a shifting of the lambda maximum of the S(λ) functions to longer wavelengths occurs. For instance, in the lobster the peak of the ERG S(λ) function is at 525 nm (Kennedy and Bruno, 1961), whereas the peak of the difference spectrum for bleaching of lobster visual pigment is at 500 nm (Wald and Hubbard, 1957; Bruno *et al.,* 1977). Similarly, in the crayfish the species visual pigment λ max appears to be at 530–535 nm (Goldsmith, 1978a) whereas the peak of the ERG S(λ) function is at 565–570 nm (Kennedy and Bruno, 1961; Goldsmith and Fernandez, 1968; Wald, 1968), a dramatic shift of about 35 nm towards the red. These bathochromic shifts in the peaks of the S(λ) functions from the peaks of the species rhodopsins have been attributed to the presence of distal screening pigment granules in some Crustacea (crayfish: Goldsmith, 1978b; crab *L. variegatus:* Stowe, 1980). It is quite conceivable that P487 overlaid by screening pigment(s) could cause a broadening of the S(λ) functions in blue-green in the purple land crab.

The purple land crab *G. lateralis* generally inhabit tropical coastal hammocks (Bliss *et al.*, 1978). For protection the land crab burrows into the sand; it never wanders too far away from the safety of its burrow. The crab is active under environmental conditions of low ambient light, temperatures from about 18.5°C to 30°C, and high humidity. Unlike the giant crab, *Cardisoma guanhumi*, *G. lateralis* is not primarily nocturnal and has been observed to be active in the subdued illumination under heavy vegetation during the day. For effective functioning in a such an environment, a medium sensitivity, low threshold receptor with maximal sensitivity in the green would be ideal for the purple land crab. The presence of a blue receptor would enable the crab to discriminate open space from closed space. Our data suggest that indeed *G. lateralis* possesses its highest sensitivity in the blue-green region of the spectrum.

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